

Amendments to the Claims

The listing of claims will replace all prior versions and listings of claims in the application.

1. (currently amended) A method for obtaining human erythropoietin comprising (a) culturing mammalian cells which express recombinant human erythropoietin in cell expansion culture medium and (b) culturing said mammalian cells in culture medium ~~comprising insulin, wherein~~ consisting of DMEM (Dulbecco's modified Eagle's medium), F12 medium, insulin and one or more additives selected from the group consisting of NaHCO₃, sugars, ethanolamine, pyruvate, amino acids and mixtures thereof ~~said cell expansion medium and said medium containing insulin contain less than 2 grams per liter of glucose.~~

2. (original) The method of claim 1, wherein said cells are selected from the group comprising CHO, COS, BHK, Namalwa, and HeLa.

3. (original) The method of claim 2, wherein said cells comprise CHO cells.

4. (previously presented) The method of claim 1, wherein said culture medium (b) comprises greater than 1 mg insulin per liter of culture medium.

5. (previously presented) The method of claim 1, wherein said culture medium (b) comprises less than 20 mg insulin per liter of culture medium.

6. (previously presented) The method of claim 1, wherein said culture medium (b) comprises fetal calf serum-free media.

7. (previously presented) The method of claim 1, further comprising:

- (c) separating supernatant comprising EPO and insulin from step (b) from cells;
- (d) concentrating supernatant of step (c); and
- (e) freezing concentrated product of step (d).

8. (previously presented) The method of claim 7, wherein media is added to separated cells of step (c) and said cells are cultured.

9. (previously presented) The method of claim 7, wherein supernatant of said step (c) is concentrated approximately 50 to 150 fold.

10. (previously presented) The method of claim 7, wherein supernatant of said step (c) is concentrated about 100 fold.

11. (previously presented) The method of claim 7, wherein said step (d) comprises using a tangential filtration system through membranes with a molecular weight cut-off of about 3,000 Daltons.

12. (previously presented) The method of claim 7, further comprising (f) sterile filtering the concentrated product of step (e) through membranes with pores of diameters of about 0.2 μm .

13. (previously presented) The method of claim 1, wherein said culture medium (b) comprises about 10 mg insulin per liter of culture medium.

14. (canceled)

15. (new) The method of claim 1, wherein said sugars are selected from the group consisting of glucose, lactose, galactose and mixtures thereof.

16. (new) The method of claim 1, wherein said pyruvate is sodium pyruvate.

17. (new) The method of claim 1, wherein said amino acids are selected from the group consisting of glutamine, tryptophan, asparagine, serine and mixtures thereof.

18. (new) The method of claim 1, wherein said culture medium (b) contains NaHCO₃, sugars, ethanolamine, sodium pyruvate and amino acids as additives.

19. (new) The method of claim 18, wherein said culture medium (b) contains Iscove's DMEM, HAM's F12 medium, insulin and NaHCO₃, glucose, lactose, galactose, ethanolamine, sodium pyruvate, glutamine, tryptophan, asparagine and serine as additives.

20. (new) The method of claim 1, wherein said DMEM is Iscove's DMEM and wherein said F12 medium is HAM's F12 medium.